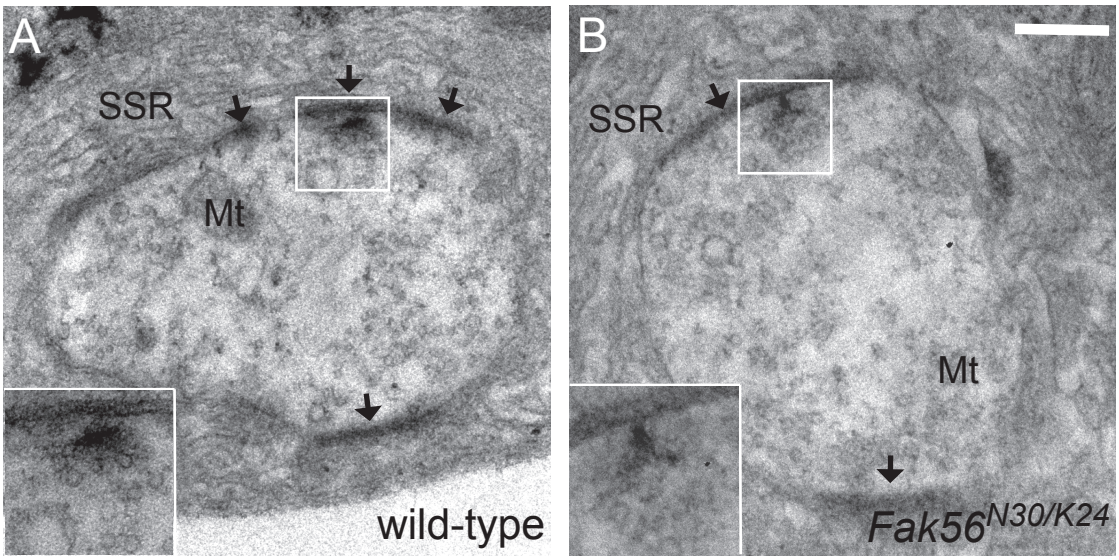

C

	wild-type (n=30)	*Fak56*$^{N30/K24}$ (n=30)	P-value
Single bouton area (μm^2)	2.61 ± 0.55	2.60 ± 0.27	0.940
Bouton perimeter (μm)	6.02 ± 0.53	6.04 ± 0.35	0.976
Active zone / Bouton	4.15 ± 0.50	3.6 ± 0.29	0.349
Total active zone length (μm) / Bouton	1.95 ± 0.31	2.05 ± 0.18	0.776
Total active zone length (μm) /Bouton perimeter (μm)	0.30 ± 0.03	0.34 ± 0.02	0.279
T bar number / Bouton	1.45 ± 0.31	1.27 ± 0.20	0.623
Bouton area (μm^2) /Active zone	0.60 ± 0.10	0.79 ± 0.08	0.150
T bar number /Active zone	0.32 ± 0.07	0.37 ± 0.05	0.642
Vesicle number /Bouton area (μm^2)	81.86 ± 9.06	84.46 ± 7.62	0.827

Additional file 3. (A-B) Electron micrographs of cross-sections through a type I bouton of muscle 6/7 in wild-type and *Fak56*$^{N30/K24}$ larvae. Squares show active zones with a T bar, which are enlarged in lower-left corner. Subsynaptic reticula (SSR), active zones (arrow), and mitochondria (Mt) are indicated. Scale bar, ~2μm. (C) Features of the synaptic ultrastructure were quantified, and no significant difference in these parameters was found between wild-type and *Fak56*$^{N30/K24}$. Dissected larval body walls (including the CNS and motor axons) were fixed at RT for 30 minutes, followed by 4°C overnight in modified Trump's fixative (0.1 M sodium cacodylate buffer, 1% glutaraldehyde, and 4% formaldehyde). The fixed specimens were rinsed three times in 0.1 M sodium cacodylate buffer for 10 minutes, post-fixed for 30 minutes with 2% osmium tetroxide in

0.1 M sodium cacodylate buffer, rinsed three times for 10 minutes in 0.1 M sodium cacodylate buffer, and finally rinsed five times in ddH_2O for 10 minutes. The muscle 6/7 in the A3 segment was knife-dissected out, and specimens were then stained *en bloc* in 2% aqueous uranyl acetate for 20 minutes, dehydrated in a graded ethanol series, and subsequently set into the Spurr's embedding medium. Thin sections (90 nm) were stained with uranyl acetate and lead citrate, and images were viewed on a Tecnai G2 Spirit TWIN electron microscope (FEI Company) and captured on a Gatan CCD Camera (794.10.BP2 MultiScan™). TEM data were quantified by MetaMorph V6.3r7 (Molecular Devices).